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Postharvest Biology and Technology 41 (2006) 22–31

Postharvest Biology and Technology

www.elsevier.com/locate/postharvbio

Aroma volatile emission and expression of 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase genes in pears treated with 2,4-DP

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Received 8 October 2005; accepted 8 March 2006

Abstract

Effects of the synthetic auxin 2,4-dichlorophenoxy-propionic acid (2,4-DP) on 1-aminocyclopropane-1-carboxylate (ACC) synthase and oxidase gene expression in 'La France' and aroma production in 'Bartlett' pears (*Pyrus communis* L.) were investigated. In non-stored, non-treated 'La France' fruit, the accumulation of ACC synthase (ACS) and ACC oxidase (ACO) transcripts was not observed. In 2,4-DP treated 'La France' fruit, the level of mRNAs hybridized with *ACS4* probe increased strongly while *ACS1*, *ACS3*, and *ACO1* mRNA levels were similar between 2,4-DP treated fruit and stored non-treated fruit. The result indicates that *ACS4* may be an ACC synthase gene which is induced by auxin in pears. Thirty-eight volatile compounds were detected from 'Bartlett' pears. The composition and amount of aroma volatiles were similar between 2,4-DP treated fruit and stored non-treated fruit. Esters were the most prevalent compounds and butyl-, ethyl-, and hexyl acetate were produced in the largest amounts. In non-stored, non-treated fruit, aldehydes constituted a high percentage of the total volatiles detected, although the amount of total volatiles detected was relatively low. Internal browning in 2,4-DP treated 'Bartlett' fruit developed on the tree within 30 days of application. Possible effects of pre-harvest ethylene, carbon dioxide, and temperatures, are discussed.

Keywords: Auxin; Ethylene; *Pyrus communis* L.; Ripening

1. Introduction

European pears (*Pyrus communis* L.) have a climacteric ripening pattern, but with some exceptions including 'Bartlett' (Wang et al., 1971), most cultivars ripen only after exposure to a sufficient duration of chilling (Bower et al., 2003). Chilling results in enhanced ethylene production and accelerated ripening when fruit are held at relatively warm temperatures after cold storage (Blankenship and Richardson, 1985; Larrigaudiere and Vendrell, 1993). The ethylene biosynthetic pathway proceeds from methionine to ethylene via 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase (Adams and Yang, 1979). Activity of these two enzymes can be influenced by a number of factors including temperature and exogenous chemicals (Abeles et al., 1992). Increased ACC synthase and ACC oxidase activity enhances ethylene synthesis and provides a means for autocatalysis of ethylene during climacteric fruit ripening (Abeles et al., 1992). Recently, cDNAs encoding these enzymes have been isolated in many plants including pear fruit (Sato and Mizuno, 2003). These enzymes are encoded by multigene families and expression of specific genes is regulated by a number of factors. The synthetic auxin 2,4-dichlorophenoxy-propionic acid (2,4-DP) has been demonstrated to prevent pre-harvest abscission of apples, but application of the material at high concentrations can induce ethylene production and fruit ripening (Kondo and Hayata, 1995). We showed the induction of ripening capacity in 'La France' pear is influenced by the 2,4-DP (Kondo and Takano, 2000; Kondo et al.,

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doi:10.1016/j.postharvbio.2006.03.004

2004). Application of 2,4-DP promoted ethylene production in tree-attached pear fruit and this response accelerated the onset of fruit ripening. For Japanese pears, the expression of ACC synthase genes varies among cultivars according to ethylene production patterns (Itai et al., 1999). The ripening capacity in European pears also varies among cultivars. However, how ethylene biosynthetic gene expression is influenced by 2,4-DP is unclear. In this study, in order to clarify the effect of 2,4-DP on the ripening capacity of pears, the effect of 2,4-DP on ethylene biosynthetic gene expression was examined, using 'La France' pears.

Ethylene regulates production of fruit aroma volatiles, a critical component of fruit quality. Our previous report (Kondo et al., 2005) showed that volatile production following application of the ethylene action inhibitor 1-methylcyclopropene (1-MCP) was lower compared to untreated controls. Although 2,4-DP treatment induces ethylene production in tree-attached pear fruit, the effect of 2,4-DP on aroma volatiles is unclear. Furthermore, little information exists regarding volatile production at harvest and after storage for pear fruit. Our previous reports (Kondo and Takano, 2000; Kondo et al., 2004) demonstrated that ethylene production from 'La France' pears induced by 2,4-DP application was accelerated by an increase in ambient temperature after the application, and the increased ethylene production and higher temperature contribute to enhanced fruit ripening. This result suggests that 2,4-DP application may be more practical in cultivars harvested earlier than 'La France', and the effects of 2,4-DP on aroma volatile production may also be clearer. Therefore, aroma volatile production in 'Bartlett' pears treated by 2,4-DP was characterized.

2. Materials and methods

2.1. Chemicals

2,4-Dichlorophenoxy-propionic acid (a.i. 10.0%) was obtained from Nissan Chemical Industries, Ltd. (Tokyo). 1-Aminocyclopropane-1-carboxylic acid was purchased from Sigma–Aldrich Co. (Milwaukee, WI).

2.2. Plant materials

Nine randomly selected 15-year-old 'La France' pear trees, grafted onto 'Quince C' (*Cydonia oblonga* Mill.) rootstock, growing in an open field at Prefectural University of Hiroshima were used in 2003. Each tree was trained as a central leader and planted in a single row from east to west with spacing of 3.0 m × 4.0 m. Furthermore, 12 randomly selected 34-year-old 'Bartlett' pear trees, grafted onto seedling rootstock, at the Columbia View Experimental Plots in Wenatchee, Washington were used in 2004. Each tree was trained as a central leader and planted in a single row from east to west with spacing of 2.5 m × 4.5 m.

For 'La France', six groups of three trees each were identified. Treatments to each group are described in Table 1. A 90 $\mu l\,l^{-1}$ 2,4-DP solution was applied by spraying whole trees to drip 130 days after full bloom (DAFB). Thirty fruit (10 from each tree) were sampled at 130 or 180 DAFB from groups one and two, and from groups three and four at 180 or 190 DAFB. In the fifth and sixth test group (stored non-treated), 30 fruit each (10 from each tree) from the non-treated trees were harvested at 160 DAFB and held at 20 °C and 90% RH for 20 or 30 days. For 'Bartlett', four test groups of three trees each were used. Treatments to each group are described in Table 2. For the first two groups, 12 fruit from each tree (36 fruit) were harvested every 3–4 days from 130 to 145 DAFB. For groups two and three, 2,4-DP at 90 $\mu l\,l^{-1}$ was applied by spraying whole trees to drip 103 DAFB. For groups three and four, 180 fruit (60 fruit from each tree) were harvested 130 DAFB and held in the dark at 20 °C and 90% relative humidity (RH). Thirty-six fruit were sampled at 3–4 days intervals over 15 days in storage. Ethylene and carbon dioxide production, volatile compound emission, firmness, peel hue value, and malic acid concentration were measured.

2.3. Northern blot hybridization

cDNAs of *ACC synthase* (*ACS*) *1*, *3*, and *4* and *ACC oxidase* (*ACO*) *1* were a gift from Dr. H. Murayama at Yamagata university. The fragments used for probes were from bp 142 to bp 1566 of the *ACS 1* clone with accession number AY388987, from bp 288 to bp 790 of the *ACS 3* clone with AY388988, from bp 148 to bp 641 of the *ACS 4* clone with AF386518, and from bp 1 to bp 1542 of the *ACO 1* clone with X87097 (Lelievre et al., 1997; El-Sharkawy et al., 2004). The specificity of each probe was confirmed by Southern blot analysis. Each cDNA was used as a probe on gel blots of plasmids cut with *Eco*RI and *Hind*III containing other cDNAs, however, cross-hybridization was not observed

Table 1
Test groups on 'La France'

Treatment	2,4-DP ($\mu l\,l^{-1}$)	Treatment description
(1) Non-stored, non-treated (130)	0	Non-treated with 2,4-DP and sampled at 130 DAFB
(2) Non-stored, non-treated (180)	0	Non-treated with 2,4-DP and sampled at 180 DAFB
(3) 2,4-DP treated (180)	90	Treated with 2,4-DP at 130 DAFB and sampled at 180 DAFB
(4) 2,4-DP treated (190)	90	Treated with 2,4-DP at 130 DAFB and sampled at 190 DAFB
(5) Stored non-treated (20)	0	Non-treated fruit harvested at 160 DAFB and stored for 20 days
(6) Stored non-treated (30)	0	Non-treated fruit harvested at 160 DAFB and stored for 30 days

Table 2
Test groups on ‘Bartlett’

Treatment	2,4-DP($\mu l\, l^{-1}$)	Treatment description
(1) Non-stored, non-treated	0	Non-treated with 2,4-DP
(2) 2,4-DP treated	90	Treated with 2,4-DP at 103 DAFB
(3) Stored 2,4-DP treated	90	Treated with 2,4-DP at 103 DAFB, harvested at 130 DAFB, and stored
(4) Stored non-treated	0	Non-treated fruit harvested at 130 DAFB and stored

(data not presented). The primers used for the probe amplification are shown in Table 3.

Total RNA was isolated from the pulp sample according to the method of Loulakakis et al. (1996). The Northern blot analysis was carried out using a previously reported method (Kondo et al., 2002). Total RNA of 20 μg was separated by electrophoresis in a 1.2% agarose gel containing 0.66 M formaldehyde and then it was transferred to a nylon membrane. Probes were labeled with digoxigenin (DIG), using the PCR DIG probe synthesis kit (Boehringer Mannheim, Mannheim, Germany). Northern membranes were hybridized according to the manufacturer's instructions. Hybridization occurred for 16 h at 50 °C within a hybridization buffer of DIG Easy Hyb (Boehringer Mannheim, Germany). After the membranes were washed twice in (2 × SSC) 150 mM NaCl and 15 mM trisodium citrate, pH 7.0 and 0.1% (w/v) SDS at room temperature for 5 min each, they were washed twice in 0.1 × SSC, 0.1% SDS at 68 °C for 15 min each.

2.4. Analysis of aroma volatile compounds, ethylene, and carbon dioxide

Volatile compound analysis was carried out according to the method previously described by Mattheis et al. (1991). Six pears per treatment (three replications) were sealed in glass jars (4 l) and the jars purged with compressed air that had been passed through traps containing activated charcoal and molecular sieve. Jars were purged at 50 ml min^{-1} for 3 h, then volatile compounds exiting the jars were collected onto 50 mg Tenax GC (Alltech Assoc., Deerfield, IL) packed into glass traps. Using an automated thermal desorption and cryofocusing autosampler (Aerotrap, Techmar Associates, Cincinnati, OH), the absorbed volatile compounds in the trap were introduced into a gas chromatograph with a mass selective detector (GC-MSD) (HP 5890, 5971A, Hewlett Packard, Avondale, PA) with a DB-1 (J&W Scientific, Folsom, CA) fused silica capillary column (60 m × 0.32 mm i.d.). The column oven temperature was increased from 35 to 225 °C at a rate of 8 °C min^{-1} after held at 35 °C for 3 min. Compounds were quantified by selected ion monitoring for base peaks with concentrations calculated using authentic standards. Ethylene and carbon dioxide also were sampled with volatile compounds and analyzed by a gas chromatograph (HP 5890, Hewlett-Packard, Avondale, PA) equipped with a glass column (610 mm × 3.2 mm i.d.) packed with Porapak Q (80–100 mesh) (Supelco, Bellefonte, PA) and flame ionization detector for ethylene; thermal controlled detector for carbon dioxide. Oven, detector, and injection temperature were 50, 200, and 100 °C, respectively. N_2, H_2, and air flows were 25, 25, and 300 ml min^{-1}, respectively.

Table 3
Primers used for probe amplification

Primer	Sequence	Accession no.
ACS1-F	5′-ATGTGCATGTTATCCAGAAACGCCA-3′	AY388987
ACS1-R	5′-TCATCTACCGTGGATAGGACAGCGG-3′	AY388987
ACS3-F	5′-TCTGTTGCAAAACCACTTGGA-3′	AY388988
ACS3-R	5′-AATAGTACCCCTCTCACTCTC-3′	AY388988
ACS4-F	5′-CTTGGTTGAAG AGTGGATTAG-3′	AF386518
ACS4-R	5′-ATGATCAAGCCCTTGACATTG-3′	AF386518
ACO1-F	5′-ACTCCATTGTCATAAACTTAG-3′	X87097
ACO1-R	5′-AGCTC AGGTAGTTGCAACAAG-3′	X87097

Fruit firmness was measured on two pared surfaces of each pear using a fruit tester with an 8 mm probe (Mohr and Associates, Richland, WA). Malic acid was measured by titrating 10 ml juice with 0.1 M KOH using an autotitrator (Radiometer, Copenhagen, Denmark). The color of all fruit in each sample group was recorded as $L^*I^*E^*$ using a chromameter (CR-200; Minolta Co., Ltd., Osaka, Japan) calibrated to a white plate. Values were converted to hue^o using the procedure of McGuire (1992).

2.5. Analysis of ACC synthase, ACC oxidase activity, and ACC concentration

ACC extraction and analysis were estimated according to the previous report (Kondo et al., 1991). Analysis of ACC synthase was performed with a modification of the method previously described by Jiang et al. (1994). Samples were homogenized with 3 ml g^{-1} of ice-cold extraction buffer [50 mM Tris–HCl buffer containing 1 mM dithiothreitol (DTT) and 1 mM phenylmethylsulfonyl fluoride (PMSF) at pH 8.2]. The homogenate was centrifuged at 10,000 × g_n for 20 min at 1 °C. The supernatant was desalted through a Sephadex G25 column (PD-10). For determination of the activity of ACC synthase, 0.9 ml of desalted extract was placed in a glass tube with 50 mM SAM, 4 mM pyridoxal phosphate, and 50 mM Tris–HCl buffer containing 1 mM DTT and 1 mM PMSF at pH 8.2 of 0.1 ml. For analysis of ACC oxidase, each excised flesh sample of 2 g was dipped into 15 ml of 0.4 M mannitol and 10 mM phosphate buffer, containing 5 mM ACC in 50 ml flask. After the ethylene in

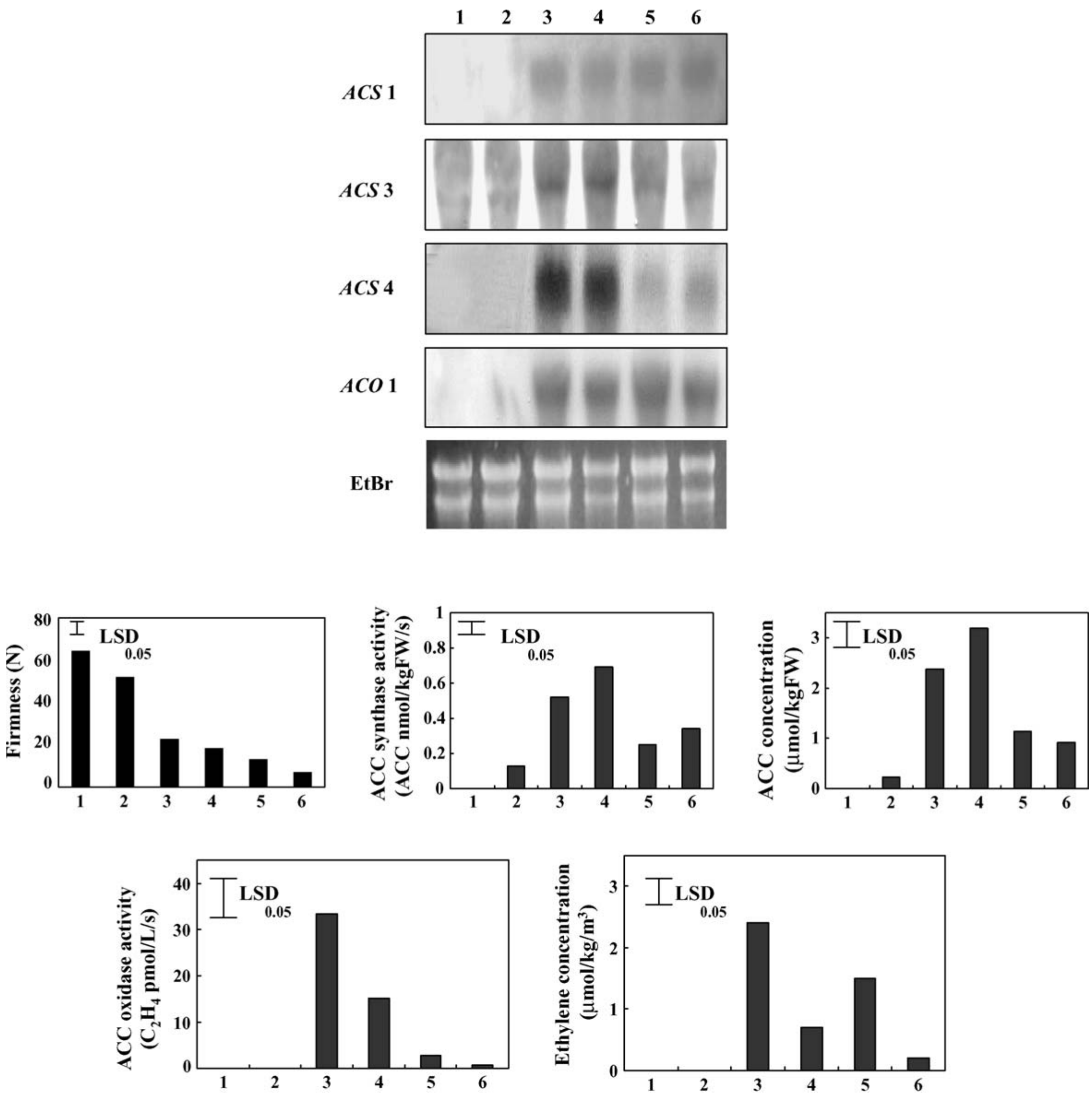

Fig. 1. Northern blots, firmness, ACC synthase activity, ACC concentration, ACC oxidase activity, and ethylene concentration from 'La France' fruit flesh. For RNA blot analysis, total RNAs (20 µg) were hybridized with *ACS 1*, *ACS 3*, *ACS 4*, and *ACO 1*. The analysis was repeated three times. Bottom panel shows the ethidium bromide-stained gel as a loading control. Data of firmness are the means of 12 fruit. The analyses of ACC synthase activity, ACC concentration, ACC oxidase activity, and ethylene concentration were repeated three times. (1) non-stored, non-treated (130), (2) non-stored, non-treated (180), (3) 2,4-DP treated (180), (4) 2,4-DP treated (190), (5) stored non-treated (20), (6) stored non-treated (30).

the sample was removed by low pressure for 5 min, the flask was closed and then was placed at 25 °C for 2 h. Ethylene in the head space of the syringe was determined by GC-FID (model GC-380; GL Sciences, Tokyo; Column: Porapak Q, 2.2 mm i.d. × 2.0 m).

2.6. Statistical analysis

In Figs. 1–4, the SAS ANOVA procedure was used to citate treatment effects, average separation was analyzed by Fisher's least significant difference ($p \leq 0.05$), and data were presented as means ± S.E. in Table 4 and Fig. 5 (SAS, Cary, NC).

3. Results

3.1. ACC synthase and ACC oxidase gene expression, ACC synthase and ACC oxidase activity, ACC and ethylene concentration in 'La France' pears

In 'La France' pears, the expression of *ACS 1*, *3*, *4*, and *ACO 1* was not observed in non-stored, non-treated fruit harvested 130 and 180 DAFB with high firmness values (Fig. 1). In contrast, *ACS 1*, *3*, *4*, and *ACO 1* were detected in 2,4-DP treated fruit, and in stored non-treated fruit. The expression of *ACS 1*, *ACS 3*, and *ACO 1* was similar between 2,4-DP treated and stored non-treated fruit. However, the expression of *ACS*

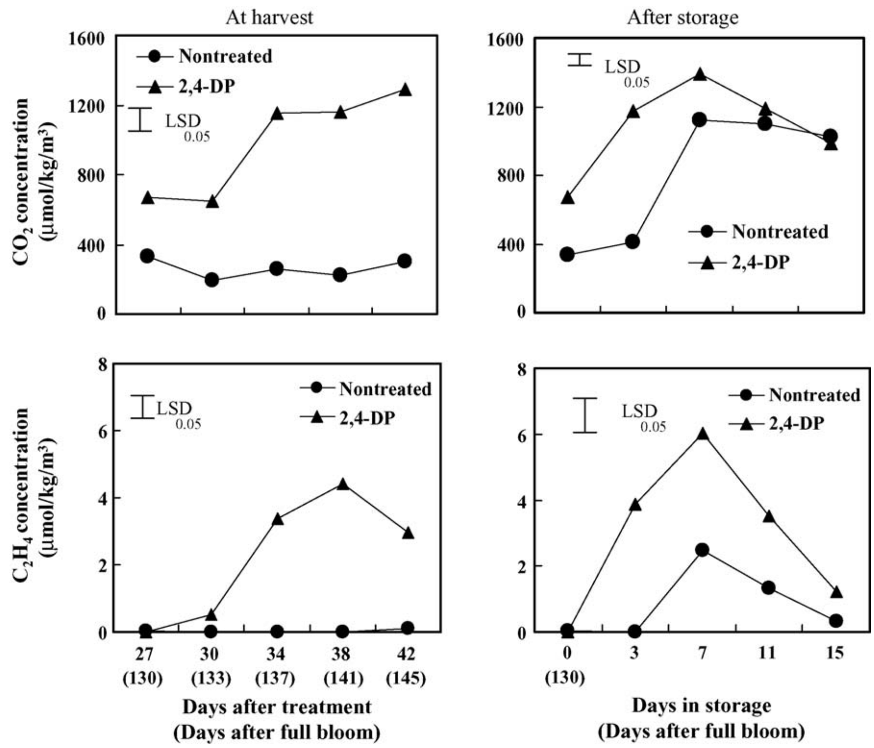

Fig. 2. Effect of 2,4-DP treatment on ethylene and carbon dioxide production in 'Bartlett' pears. See Table 2 for legend. Data are the means of three replications of six fruit.

4 in 2,4-DP treated fruit was much higher than the expression in stored non-treated fruit. Although ACC synthase activity and ACC concentration were high at 2,4-DP treated fruit, ACC oxidase activity and ethylene production were high at 2,4-DP treated (180) fruit only. However, firmness of stored non-treated fruit was the lowest of all treatments.

3.2. 'Bartlett' pear volatile production at harvest and after storage

Application of 2,4-DP promoted ethylene (C_2H_4) and carbon dioxide (CO_2) production by 'Bartlett' pears (Fig. 2). Production of C_2H_4 and CO_2 by 2,4-DP treated fruit increased during the 30 days after treatment compared to non-treated controls. Although both C_2H_4 and CO_2 production increased in both stored 2,4-DP treated and stored non-treated fruit, the concentrations in 2,4-DP treated fruit were higher. C_2H_4 concentrations decreased after 7 days in storage in both stored 2,4-DP treated and stored non-treated fruit.

Thirty-eight volatile compounds were detected from pears regardless of 2,4-DP treatment although amounts of each compound varied among treatments (Table 4). These volatiles were of five groups: alcohols (1-butanol, ethanol, 1-hexanol, 2-methyl-1-butanol, 2-methyl-1-propanol, 1-pentanol, and 2-propanol), esters (butyl acetate, butyl butyrate, butyl hexanoate, butyl 2-methylbutyrate, butyl propanoate, ethyl acetate, ethyl butyrate, ethyl 2,4-decadienoate, ethyl hexanoate, ethyl propanoate, hexyl acetate, hexyl butyrate, hexyl hexanoate, hexyl 2-methylbutyrate, methyl butyrate, methyl 2,4-decadienoate, 2-methylbutyl acetate, 2-methylpropyl acetate, pentyl acetate, propyl acetate, and propyl butyrate), ketones (6-methyl-5-hepten-2-one), aldehydes (benzaldehyde, butanal, decanal, hexanal, heptanal, nonanal, octanal, and pentanal), and the sesquiterpene hydrocarbon α-farnesene. Ester and alcohol production were higher than those of any other volatile groups except for non-stored, non-treated fruit for which aldehyde production was highest of all groups of volatiles. Ethanol and 1-butanol were primary volatiles in the alcohol group, and butyl acetate, ethyl acetate, and hexyl acetate were foremost in the ester group. Aldehyde production levels were highest in the non-stored, non-treated fruit, and decanal, benzaldehyde, and hexanal were primary volatiles in the aldehyde group. Production of alcohols, esters, ketones, and aldehydes increased during ripening of 2,4-DP treated fruit during storage (Fig. 3). The increases of these volatiles were not observed in non-stored, non-treated fruit. α-Farnesene production in 2,4-DP treated fruit was higher compared to non-stored, non-treated fruit, but production did not increase with days of ripening. In both stored 2,4-DP treated and stored non-treated fruit, alcohol and ester production increased with days of ripening up to 15 days in storage, and ketone and aldehyde production increased up to 11 days in storage. In general, these concentrations were higher for 2,4-DP treated fruit compared to stored non-treated fruit.

Firmness and hue value decreased with days of ripening after harvest except for non-stored, non-treated fruit (Fig. 4), and values were lower for 2,4-DP treated fruit. Although

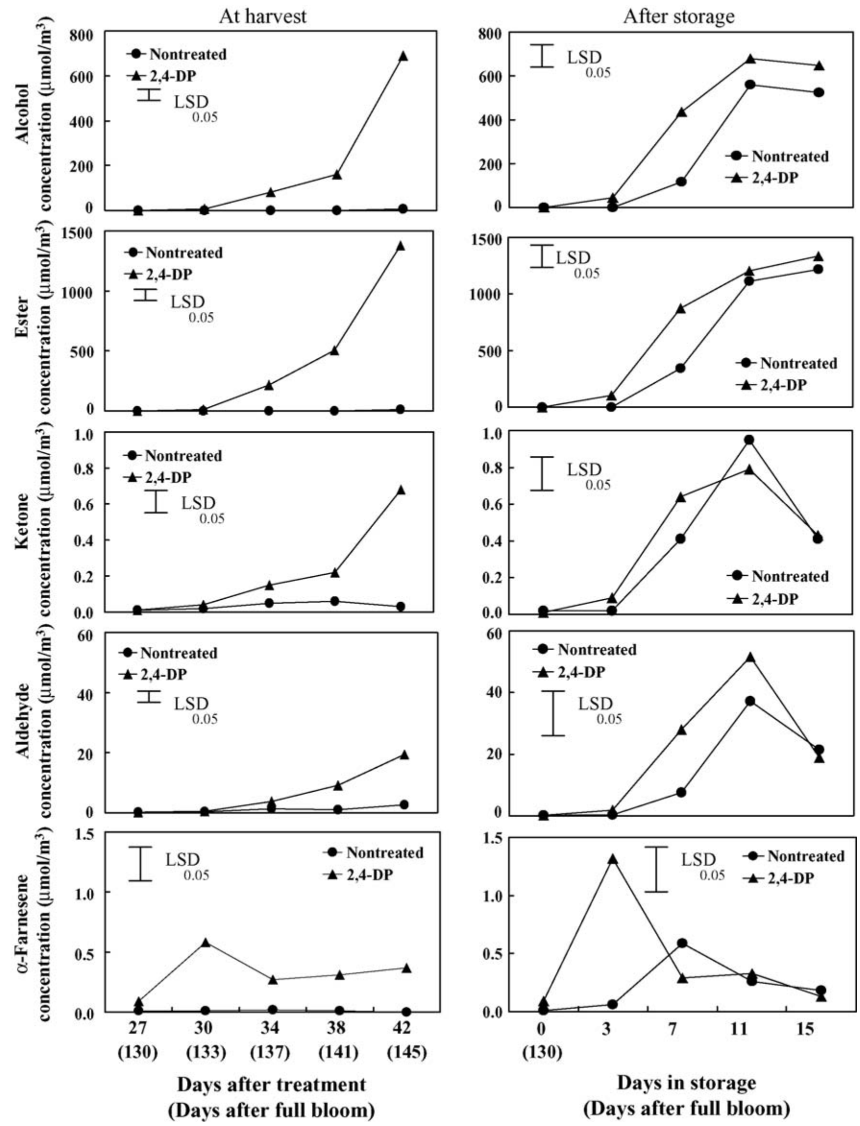

Fig. 3. Effect of 2,4-DP treatment on total alcohol, ester, ketone, aldehyde, and α-farnesene production in 'Bartlett' pears. See Table 2 for legend. Data are the means of three replications of six fruit.

malic acid concentrations decreased with days of ripening, there was not a significant difference between treatments. Internal browning developed in 2,4-DP treated fruit after treatment and the incidence of internal browning increased up to the last harvest 42 days after treatment (Fig. 5).

4. Discussion

Up to 10 different ACC synthase genes have been reported in plants (Sato and Mizuno, 2003) and auxin-induced ACC synthase gene varies among plants. For instance, *ACS3* in tomato (*Lycopersicon esculentum* Mill.), *ACS1* in apple [*Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf.], and *ACS4* in *Arabidopsis* are induced by auxin treatment (Sato and Mizuno, 2003). Our results indicate that *ACS4* in 'La France' pear, may be induced by the synthetic auxin 2,4-DP and *ACS4* transcript accumulation may contribute to the increase of ACC synthase activities and ACC concentrations in 2,4-DP treated fruit. In tomatoes, auxin induction of a gene encoding ACC synthase, *ACS5*, has been shown (Coenen et al., 2003). In pears and tomatoes, it has been shown that the effect of exogenous auxin on fruit ripening differed with the application method (Frenkel and Dyck, 1973; Vendrell, 1985). That is, although both surface and vacuum infiltration treatment stimulated ethylene production, the latter inhibited fruit ripening. This result suggests that a difference in intake of auxin into the fruit may influence the ripening. Therefore, the expression of each gene encoding ACC synthase and ACC oxidase should be observed at short time intervals after auxin application. In Japanese pears, *ACS1* was specific to cultivars which have high levels of ethylene, *ACS2* was specific to cultivars which produced moderate amounts of ethylene, and *ACS3* mRNA was detected at low levels in all cultivars,

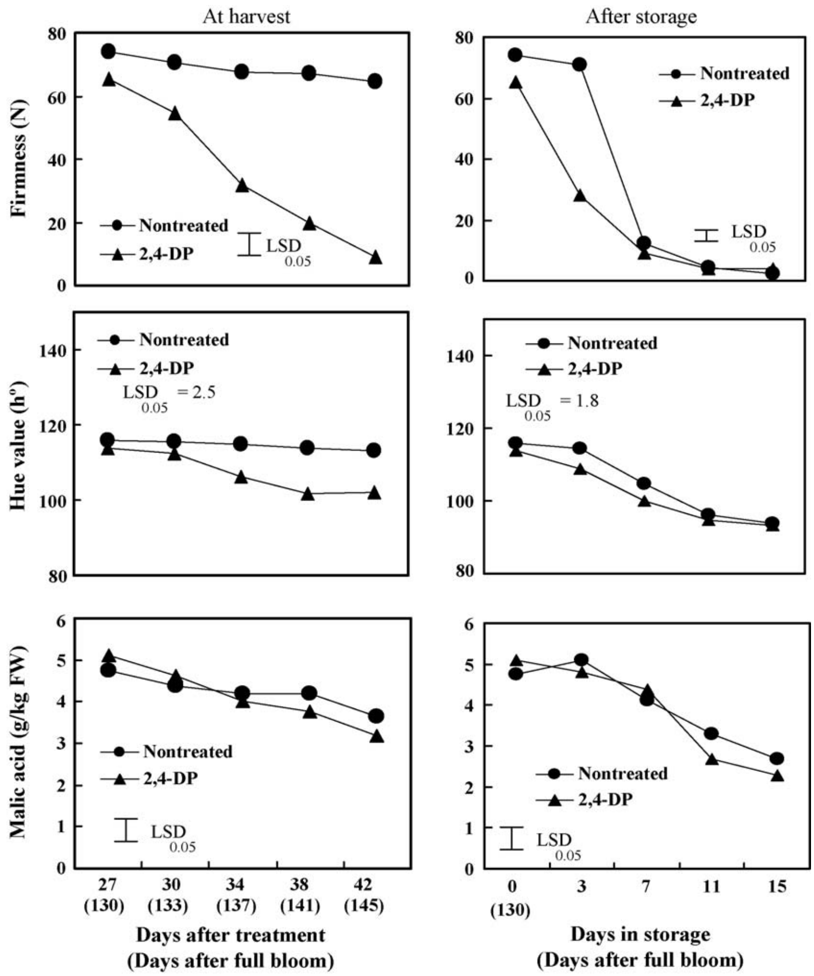

Fig. 4. Effect of 2,4-DP treatment on fruit firmness, color, and malic acid concentrations in 'Bartlett' pears. See Table 2 for legend. Data are the means of 36 fruit.

regardless of the difference of ethylene levels (Itai et al., 1999, 2003). Also in apples and peaches (*Prunus persica* L.), *ACS1* is the primary gene responsible for ethylene production during ripening (Harada et al., 2000; Mathooko et al., 2001). In addition, also in pear, *ACS1* may link ethylene production to the onset of climacteric ripening, because the expression of *ACS1* in stored non-treated fruit was stronger than that of *ACS3* and *4*. In 'Passe-Crassane' pears which require chilling temperatures to induce endogenous ethylene production, chilling significantly promoted ACC oxidase activity, and to a lesser extent ACC synthase activity (Lelievre et al., 1997). These authors also reported that the chilling-induced accumulation of ACC oxidase and ACC synthase transcripts was reduced following 1-MCP treatment which blocks ethylene receptors and retards ethylene action. Therefore, it is possible that the *ACO1* mRNA transcript is induced by chilling temperatures and ethylene. In our study, 'La France' pears were ripened at 20 °C without the chilling treatment after harvest. Regardless of this fact, the accumulation of *ACO1* mRNA was not different between 2,4-DP treated and non-treated fruit, although ACC oxidase activity and ethylene production from 2,4-DP treated fruit were higher than those from non-treated fruit. The result that the *ACO1* mRNA transcript was not observed in non-stored, non-treated fruit suggests that *ACO 1* also is related to induction of ripening in 'La France' pears. In melons, ACC oxidase cDNAs for *ACS 1*, *2*, and *3* have been confirmed (Sato and Mizuno, 2003). Therefore, it is possible that other *ACO* genes may be associated with the high ACC oxidase activities in 2,4-DP treated fruit. In summary, *ACO1* and *ACS1* are not regulated at the transcription level by 2,4-DP but may regulate fruit ripening developmentally.

Significant increases in ethylene production by 'La France' and 'Bartlett' pears were observed at 40 and 30 days, respectively, after 2,4-DP treatment. The difference in the duration between 2,4-DP application and the onset of ethylene production may be due to cultivar differences in development and maturation. The production of aroma volatiles in climacteric fruit is regulated by ethylene production, and compounds that stimulate (ethephon) or inhibit (aminoethoxyvinylglycine: AVG) ethylene production impact volatile production (Lalel et al., 2003; Romani

Table 4
Primary aroma volatiles detected in 'Bartlett' pears

Compound	% of total volatile compound amount (ng/kg/m^3)			
	At harvest		After storage	
	Non-treated	2,4-DP	Non-treated	2,4-DP
Alcohols				
1-Butanol	2.27 ± 0.29	3.33 ± 0.14	3.16 ± 0.16	2.27 ± 0.20
Ethanol	5.30 ± 1.56	9.76 ± 0.92	9.20 ± 0.38	14.43 ± 1.30
1-Hexanol	0.97 ± 0.16	0.82 ± 0.05	1.34 ± 0.09	0.97 ± 0.12
Others	6.22 ± 2.97	2.34 ± 0.16	0.62 ± 0.03	1.15 ± 0.19
Esters				
Butyl acetate	4.35 ± 2.88	25.92 ± 0.23	30.06 ± 1.40	24.94 ± 1.38
Butyl butyrate	0.09 ± 0.02	0.27 ± 0.06	0.53 ± 0.09	0.16 ± 0.01
Ethyl acetate	3.36 ± 0.34	30.68 ± 0.48	28.11 ± 3.00	28.17 ± 2.97
Hexyl acetate	2.74 ± 0.95	16.48 ± 0.86	17.08 ± 0.17	16.95 ± 0.61
Pentyl acetate	0.33 ± 0.14	1.90 ± 0.08	1.68 ± 0.13	1.36 ± 0.06
Propyl acetate	0.35 ± 0.26	5.43 ± 0.81	4.92 ± 1.21	5.72 ± 0.62
Others	3.39 ± 1.66	1.47 ± 0.26	1.26 ± 0.16	1.32 ± 0.21
Ketone				
6-Methyl-5-hepten-2-one	2.48 ± 0.19	0.07 ± 0.01	0.12 ± 0.02	0.07 ± 0.01
Aldehydes				
Benzaldehyde	16.74 ± 3.87	0.37 ± 0.17	0.50 ± 0.05	0.42 ± 0.08
Decanal	18.91 ± 5.55	0.20 ± 0.15	0.21 ± 0.04	0.60 ± 0.31
Hexanal	10.28 ± 1.70	0.21 ± 0.04	0.21 ± 0.02	0.32 ± 0.04
Octanal	7.94 ± 1.32	0.13 ± 0.07	0.11 ± 0.02	0.25 ± 0.08
Others	12.39 ± 1.62	0.42 ± 0.09	0.62 ± 0.09	0.85 ± 0.02
α-Farnesene	1.80 ± 0.44	0.20 ± 0.05	0.27 ± 0.22	0.05 ± 0.00

Volatile compounds in 'Bartlett' pears were sampled from non-treated fruit stored for 7 days at 20 °C (after storage: non-treated); 2,4-DP treated fruit stored for 7 days at 20 °C after the 90 ml l^{-1} 2,4-DP solution was applied to whole trees at 103 DAFB (after storage: 2,4-DP); non-stored, non-treated fruit at 137 DAFB which were not treated with 2,4-DP (at harvest: non-treated); 2,4-DP treated fruit at 137 DAFB which were treated with the 90 ml l^{-1} 2,4-DP solution at 103 DAFB (at harvest: 2,4-DP). Data are means ± S.E. of three replications.

et al., 1983). In 2,4-DP treated fruit where the onset of ethylene production was advanced following treatment, and stored non-treated fruit, no qualitative or quantitative differences in volatiles were apparent. In contrast, in non-stored, non-treated fruit, the percentage of esters and alcohols of total volatiles detected was low relative to that of aldehydes. Acetate esters have been characterized as having floral, fruity, sweet, and perfume odors, but aldehydes have grass-like, fatty, and green odors (Paillard, 1990). This result implies that the fruit with a high aldehyde production in relation to other volatiles may be immature.

The increase of ethylene production in non-stored, non-treated, 2,4-DP treated, and stored 2,4-DP treated fruit preceded the increase in alcohol, ester, ketone, and aldehyde production. Many aroma compounds in fruit are generated from lipid metabolism. 'Bartlett' pears have lipids which are characterized by high levels of unsaturated fatty acids (Rapparini and Predieri, 2003). It is possible that production of 'Bartlett' aroma compounds increases after unsaturated fatty acids are available for ester synthesis following increased ethylene production. Esters such as methyl (*E*), (*Z*)-deca-2,4-dienoate and ethyl (*E*), (*Z*)-deca-2,4-dienoate that have been reported as character impact compounds for 'Bartlett' (Shiota, 1990) were present only in small amounts in our study. The difference may be due to the sampling method used as these compounds are not efficiently released off the Tenax traps used to collect volatile compounds. α-Farnesene was detected as the main volatile component with a 'peely fresh green' odor from 'La France' pears (Shiota, 1990). In our study, the amount of α-farnesene from 'Bartlett' was small, production did not change during ripening, but production by 2,4-DP treated fruit was greater than that of non-treated fruit. Changes in α-farnesene were highly associated with the development of superficial or senescent scald in 'd'Anjou' and 'Doyenne du Comice' pears (Chen et al., 1990; Ma and Chen, 2003). Also in apples, α-farnesene accumulation has been associated with superficial scald development (Ingle and D'Souza, 1989). The low production of α-farnesene, as well as the short storage period and relatively warm storage environment may have contributed to the lack of superficial scald development.

Internal browning was present in 'Bartlett' pears on the tree 30 days after 2,4-DP treatment. In addition, C_2H_4 and CO_2 production increased significantly 30 days after 2,4-DP treatment. It has been shown that the development of internal browning in pears is associated with C_2H_4 and CO_2 production (Bower et al., 2003; Ma and Chen, 2003). These results may indicate that internal browning in tree-attached pear fruit may be associated with endogenous C_2H_4 and CO_2 levels. However, storage temperature strongly influences the induc-

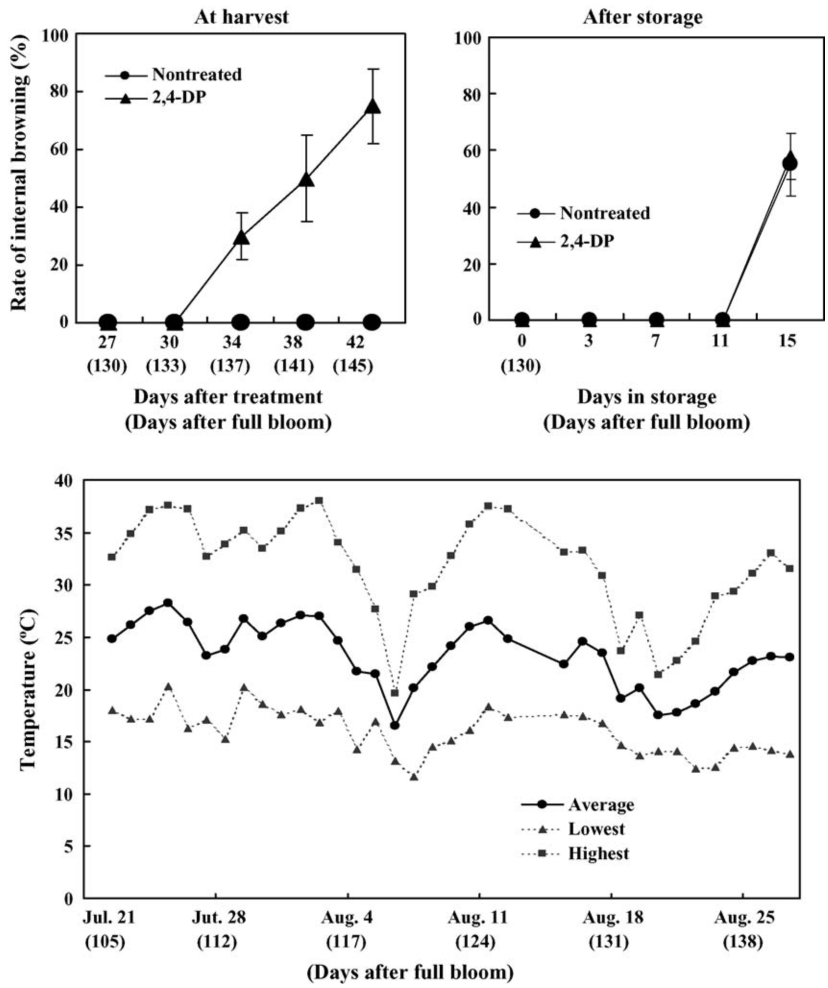

Fig. 5. 2,4-DP treatment and internal browning in ‘Bartlett’ pears. Data are from 36 fruit.

tion of other physiological disorders (Bower et al., 2003). Pears previously stored at 2 °C developed the physiological disorders when fruit were ripened at 20 °C, irrespective of exposure to ethylene, but pears stored at −1 °C ripened normally at 20 °C, regardless of exposure to ethylene. In our study, days with a maximum temperature over 30 °C occurred after 103 DAFB. The connection between temperature and internal browning also should be investigated.

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